Preclinical Pharmacology of FMPD [6-Fluoro-10-[3-(2-methoxyethyl)-4-methyl-piperazin-1-yl]-2-methyl-4H-3-thia-4,9-diaza-benzo[f]azulene]: A Potential Novel Antipsychotic with Lower Histamine H1 Receptor Affinity Than Olanzapine


Received May 10, 2005; accepted August 31, 2005

ABSTRACT

FMPD [6-fluoro-10-[3-(2-methoxyethyl)-4-methyl-piperazin-1-yl]-2-methyl-4H-3-thia-4,9-diaza-benzo[f]azulene] is a potential novel antipsychotic with high affinity for dopamine D2 (Ki = 6.3 nM), 5-HT2A (Ki = 7.3 nM), and 5-HT6 (Ki = 8.0 nM) human recombinant receptors and lower affinity for histamine H1 (Ki = 30 nM) and 5-HT2C (Ki = 102 nM) human recombinant receptors than olanzapine. Oral administration of FMPD increased rat nucleus accumbens 3,4-dihydroxyphenylacetic acid concentrations (ED50 = 6 mg/kg), blocked 5-HT2A agonist-induced increases in rat serum corticosterone levels (ED50 = 1.8 mg/kg), and inhibited the ex vivo binding of [125I]SB-258585 [4-iodo-N-[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-benzenesulfonamide] to striatal 5-HT6 receptors (ED50 = 10 mg/kg) but failed to inhibit ex vivo binding of [3H]pyrilamine to hypothalamic histamine H1 receptors at doses of up to 30 mg/kg. In electrophysiology studies, acute administration of FMPD selectively elevated the number of spontaneously active A10 (versus A9) dopamine neurons and chronic administration selectively decreased the number of spontaneously active A10 (versus A9) dopamine neurons. FMPD did not produce catalepsy at doses lower than 25 mg/kg p.o. In Fos-induction studies, FMPD had an atypical antipsychotic profile in the striatum and nucleus accumbens and increased Fos expression in orexin-containing neurons of the hypothalamus. FMPD produced only a transient elevation of prolactin levels. These data indicate that FMPD is an orally available potent antagonist of dopamine D2, 5-HT2A, and 5-HT6 receptors and a weak antagonist of H1 and 5-HT2C receptors. FMPD has the potential to have efficacy in treating schizophrenia and bipolar mania with a low risk of treatment-emergent extrapyramidal symptoms, prolactin elevation, and weight gain. Clinical trials are needed to test these hypotheses.

Olanzapine is effective in treating schizophrenia and bipolar mania (Beasley et al., 1996; Tohen et al., 2000). It is classified as an atypical antipsychotic in that it has therapeutic effects on the positive, negative, and cognitive symptoms of schizophrenia while having a low propensity to produce extrapyramidal symptoms (EPS) and prolactin elevation. However, many patients have observed weight gain associated with the treatment of olanzapine and other atypical antipsychotics (Wirshing, 2001).

It has been hypothesized that blockade of the histamine H1 receptor plays a critical role in antipsychotic treatment-emergent weight gain. Histamine has long been associated with the control of food intake. Histamine activates postsynaptic H1 receptors in the ventromedial and para-ventricular nucleus of the hypothalamus to suppress feeding (Sakata et al., 1988; Ookuma et al., 1989), and H1 knock-out mice develop diet-induced and age-related obesity (Masaki et al., 2001). In addition, a significant correlation between affinity for the human H1 receptor and weight gain has been reported for antipsychotics (Kroeze et al., 2003). Thus, an antipsychotic with a similar binding profile as olanzapine but with lower histamine H1 receptor affinity than olanzapine is a potential novel antipsychotic with lower histamine H1 receptor affinity than olanzapine.
reduced affinity for H1 receptors may retain the clinical efficacy of olanzapine but have less treatment-emergent weight gain.

We set out to discover a compound that would have a profile of pharmacological activities similar to olanzapine but with decreased activity at H1 receptors. Compounds were evaluated in a number of in vivo and in vitro tests. The inhibition of radioligand binding to neuronal receptors was examined in vitro. To evaluate in vivo D2 antagonism, rat nucleus accumbens 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations, and cocaine-induced locomotor activity were examined. To evaluate in vivo 5-HT6 and H1 antagonism, the inhibition of ex vivo binding was examined. To evaluate in vivo 5-HT2A antagonism, blockade of quipazine-induced increases in rat serum corticosterone concentrations was examined. To evaluate the potential for EPS, catalepsy induction, changes in regional Fos induction, and electrophysiological effects on dopamine neurons were examined. To evaluate the potential for treatment-emergent weight gain, Fos activation in orexin-containing cells in the rat hypothalamus was examined. In addition, serum prolactin levels were also examined. Our work has helped lead to FMPD (Fig. 1).

Materials and Methods

Radioligand Binding Assays

The assays used were standard in vitro radioligand binding assays and where possible human cloned receptors were evaluated. The assay conditions are summarized in Table 1. Radioligand binding assays were run under the conditions described in the references listed in Table 1. Separation of bound versus free ligand was achieved either by filtration through glass-fiber filters (Millipore, Billerica, MA) or by use of scintillation proximity assay (SPA) technology (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Kd values for the radioligands were determined either from saturation assays or by homologous competition assays (see Swillens, 1992). IC50 values for FMPD and olanzapine (Eli Lilly & Co., Indianapolis, IN) were determined from competition curves by nonlinear regression analysis (Motulsky, 1999). The IC50 values were converted to Kd values using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

Ex Vivo Binding

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 100 to 130 g were fasted overnight. Animals were treated orally by gavage with either vehicle (dilute lactic acid) or an antipsychotic agent for 90 min before euthanasia. Tissues were dissected quickly, frozen on dry ice, and stored at −70°C before analysis. Tissues were weighed and then homogenized in 10 volumes of the appropriate ice-cold buffer for histamine H1 ex vivo binding (50 mM monobasic sodium phosphate buffer, pH 7.4) or 5-HT6 ex vivo binding (50 mM Tris HCl buffer, pH 7.4, containing 1 mM MgCl2). Homogenates were preincubated for 10 min at 37°C to remove endogenous histamine or serotonin.

Histamine H1 Ex Vivo Binding

Ex vivo binding of the selective histamine H1 antagonist [3H]pyrilamine to rat hypothalamic homogenates was determined in triplicate. Tubes containing 100 μl of tissue homogenate were combined with 1 ml of respective buffer containing 3 nM [3H]pyrilamine (Kd = 3.9 nM). Samples were incubated for 30 min at 25°C. Nonsignificant binding was determined by the addition of 10 μM clozapine (Sigma-Aldrich, St. Louis, MO).

5-HT6 Ex Vivo Binding

Ex vivo binding of the selective 5HT6 antagonist [125I]SB-258585 (Hirst et al., 2000), which was synthesized and iodinated at Lilly Research Laboratories (specific activity = 2175 Ci/mmol, Kd = 2.3 nM), to rat striatal homogenates was determined. Triplicate tubes containing 100 μl of tissue homogenate were combined with 1 ml of respective buffer containing 0.15 nM [125I]SB-258585. Samples were incubated for 45 min at 37°C. Nonsignificant binding was determined with 1 μM clozapine.

Ex vivo samples were processed by vacuum separation-filtration using a Brandel Cell Harvester with G/F filters (Brandel Inc., Gaithersburg, MD) soaked in 0.1% polyethylenimine. Filters were washed twice with 1 ml of cold buffer and then placed in scintillation vials containing Beckman Ready Protein+ (Beckman Coulter, Fullerton, CA). Radioactivity was determined by liquid scintillation spectrometry. ED50 determinations were calculated using the Allfit statistical program for displacement binding experiments (Bymaster et al., 1996).

Nucleus Accumbens DOPAC Concentrations

Male Sprague-Dawley rats weighing 110 g were gavaged with vehicle (dilute lactic acid), olanzapine, or FMPD 90 min before sacrifice. Rat nucleus accumbens DOPAC concentrations were measured using high-pressure liquid chromatography with electrochemical detection (Fuller and Perry, 1989). Analysis of variance (ANOVA) followed by Tukey’s Honestly Significant Difference test post hoc identified significant differences between treatment groups (P < 0.05). Doses that increased DOPAC levels to 200% of control values (ED50) were calculated using a best-fit linear regression analysis.

Serum Corticosterone Concentrations

Male Sprague-Dawley rats weighing 200 to 220 g were housed in groups of five and acclimatized for 1 week. Olanzapine and FMPD were dissolved in dilute lactic acid and gavaged 1 h before administration of 2.5 mg/kg s.c. quipazine maleate (Sigma/RBI, Natick, MA). Rats were sacrificed 1 h after quipazine, trunk blood-collected, and allowed to clot. Serum corticosterone concentrations were measured in duplicate by radioimmunoassay (Corticosterone **-H-Kit; MP Biomedicals, Irvine, CA). Analysis of variance followed by Tukey’s Honestly Significant Difference test post hoc identified significant differences between treatment groups (P < 0.05). Fifty percent effective doses (ED50) were calculated using a best-fit linear regression analysis.
### TABLE 1
Summary of binding assay conditions

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Species</th>
<th>Source</th>
<th>Radioligand</th>
<th>Definition of Nonspecific Binding</th>
<th>Separation Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine D₁</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]SCH23390 (1 nM)</td>
<td>d-Butaclamol (10 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; PerkinElmer Life and Analytical Sciences&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dopamine D₂</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]Iodosiphenazine (0.1 nM)</td>
<td>Haloperidol (5 μM)</td>
<td>Filtration</td>
<td>Carpenter et al., 2002; Zgombick et al., 1991</td>
</tr>
<tr>
<td>Dopamine D₃</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]Raclopride (Raclopride or haloperidol (10 μM))</td>
<td>SPA</td>
<td>Carpenter et al., 2002; Zgombick et al., 1991</td>
<td></td>
</tr>
<tr>
<td>Dopamine D₄</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]Spirperone (Haloperidol (10 μM))</td>
<td>SPA</td>
<td>Carpenter et al., 2002; Zgombick et al., 1991</td>
<td></td>
</tr>
<tr>
<td>Dopamine D₅</td>
<td>Rat</td>
<td>Corpus striatum</td>
<td>[³H]Raclopride (0.8 nM)</td>
<td>Spiperone (0.03 μM)</td>
<td>Filtration</td>
<td>Hall et al., 1988</td>
</tr>
<tr>
<td>5-HT₁A</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]DPAT (3 nM)</td>
<td>1-NP (10 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; Zgombick et al., 1991</td>
</tr>
<tr>
<td>5-HT₁B</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]5-HT (5 nM)</td>
<td>1-NP (10 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; Zgombick et al., 1991</td>
</tr>
<tr>
<td>5-HT₁C</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]5-HT (5 nM)</td>
<td>1-NP (10 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; Zgombick et al., 1991</td>
</tr>
<tr>
<td>5-HT₂A</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]5-HT (5 nM)</td>
<td>1-NP (10 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; Zgombick et al., 1991</td>
</tr>
<tr>
<td>5-HT₂B</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]5-HT (5 nM)</td>
<td>1-NP (10 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; Zgombick et al., 1991</td>
</tr>
<tr>
<td>Histamine H₁</td>
<td>Rat</td>
<td>Hypothalamus</td>
<td>[³H]Pyridazine (10 μM)</td>
<td>Promethazine (10 μM)</td>
<td>Filtration</td>
<td>Tran et al., 1978</td>
</tr>
<tr>
<td>Histamine H₂</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]5-HT (5 nM)</td>
<td>1-NP (10 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; De Becker et al., 1993</td>
</tr>
<tr>
<td>α₁-adrenergic</td>
<td>Human</td>
<td>Whole brain</td>
<td>[³H]Prazosin (0.2 μM)</td>
<td>Yohimbine (1 μM)</td>
<td>Filtration</td>
<td>Uhlén et al., 1994</td>
</tr>
<tr>
<td>α₂-adrenergic</td>
<td>Human</td>
<td>Whole brain</td>
<td>[³H]Prazosin (0.5 μM)</td>
<td>Phentolamine (10 μM)</td>
<td>Filtration</td>
<td>Carpenter et al., 2002; Forray et al., 1994</td>
</tr>
<tr>
<td>α₂-adrenergic</td>
<td>Human</td>
<td>Whole brain</td>
<td>[³H]Prazosin (0.5 μM)</td>
<td>Phentolamine (10 μM)</td>
<td>Filtration</td>
<td>Carpenter et al., 2002; Forray et al., 1994</td>
</tr>
<tr>
<td>α₂-adrenergic</td>
<td>Human</td>
<td>Whole brain</td>
<td>[³H]MK912 (0,7 nM)</td>
<td>Yohimbine (1 μM)</td>
<td>Filtration</td>
<td>Carpenter et al., 2002; Forray et al., 1994</td>
</tr>
<tr>
<td>α₂-adrenergic</td>
<td>Human</td>
<td>Whole brain</td>
<td>[³H]RX21002 (4 nM)</td>
<td>RX21002 (10 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; Halme et al., 1995</td>
</tr>
<tr>
<td>β₁-adrenergic</td>
<td>Human</td>
<td>Whole brain</td>
<td>[³H]Iodosiphenazin (0.05 nM)</td>
<td>Iodosiphenazin (0.05 nM)</td>
<td>Filtration</td>
<td>Yamamura and Snyder, 1974</td>
</tr>
<tr>
<td>Muscarinic M₁</td>
<td>Rat</td>
<td>Cortex</td>
<td>[³H]N-Methylscopolamine (1 nM)</td>
<td>Atropine (1 μM)</td>
<td>Filtration</td>
<td>Carpenter et al., 2002; PerkinElmer Life and Analytical Sciences&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscarinic M₂</td>
<td>Human</td>
<td>Human</td>
<td>[³H]N-Methylscopolamine</td>
<td>Atropine (1 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; PerkinElmer Life and Analytical Sciences&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscarinic M₃</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]N-Methylscopolamine</td>
<td>Atropine (1 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; PerkinElmer Life and Analytical Sciences&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscarinic M₄</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]N-Methylscopolamine</td>
<td>Atropine (1 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; PerkinElmer Life and Analytical Sciences&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscarinic M₅</td>
<td>Human</td>
<td>Cloned</td>
<td>[³H]N-Methylscopolamine</td>
<td>Atropine (1 μM)</td>
<td>SPA</td>
<td>Carpenter et al., 2002; PerkinElmer Life and Analytical Sciences&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>β₂-adrenergic</td>
<td>Human</td>
<td>Whole brain</td>
<td>[³H]Iodosiphenazin (0.05 nM)</td>
<td>Iodosiphenazin (0.05 nM)</td>
<td>Filtration</td>
<td>Carpenter et al., 2002; PerkinElmer Life and Analytical Sciences&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>β₂-adrenergic</td>
<td>Human</td>
<td>Whole brain</td>
<td>[³H]Iodosiphenazin (0.05 nM)</td>
<td>Iodosiphenazin (0.05 nM)</td>
<td>Filtration</td>
<td>Carpenter et al., 2002; PerkinElmer Life and Analytical Sciences&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The assays utilizing SPA were based on those described by Carpenter et al. (2000) as adapted from the referenced filtration methods. All SPA assays used WGA-SPA beads (GE Healthcare), with the exception of the assays using [³H]Prazosin, where polyethylene-coated yttrium silicate beads (GE Healthcare) were used.

<sup>b</sup> PerkinElmer technical data sheet, RBHD2CM: Human D2 Dopamine Receptor (http://las.perkinelmer.com/content/TechnicalInfo/rbhd2_cm.pdf).

<sup>c</sup> PerkinElmer technical data sheet, RBHD44M: Human D4 Dopamine Receptor (D4.4 Variant) (http://las.perkinelmer.com/content/TechnicalInfo/rbhd44m.pdf).

<sup>d</sup> The assays utilizing SPA were based on those described by Carpenter et al. (2000).
Cocaine-Induced Locomotor Activity

Male Lister Hooded rats (Harlan Olac, Bicester, UK) weighing 180 to 250 g were used in this study. The animals were housed in groups of up to five in ventilated metal cages in a room maintained at 21 ± 1°C (relative humidity 50 ± 5%) on a 12-h light/dark cycle (lights on 7:00 AM to 7:00 PM). All of the animals had free access to food and water.

The animals were removed from the holding room and randomly assigned to treatment groups. Animals received either vehicle or test substance by the oral route immediately before being placed in the activity cages for the 30-min habituation period. After the habituation, the animals received cocaine (40 mg/kg i.p.) and were returned to the activity cages for a further 90 min.

Activity was measured as light beam interruptions per 10-min period. Comparisons between groups were carried out using a one-way ANOVA procedure for independent groups followed by a post hoc least-square means multiple comparison test within SAS Proc GLM. A probability of $P < 0.05$ was considered significant.

Catalepsy

Male Lister Hooded rats were used in the studies. The animals were housed in groups of up to five in conventional ventilated metal cages in a room maintained at 21 ± 1°C (relative humidity 50 ± 5%) on a 12-h light/dark cycle (lights on 7:00 AM to 7:00 PM). All of the rats had free access to food and water.

FMPD, olanzapine, chlorpromazine, or haloperidol was administered by the oral route, and catalepsy was determined at hourly intervals starting 1 h after dosing. Catalepsy was determined by placing the animal's front paws over a rod raised approximately 6 cm above the bench. The time for which the animal remained in this position was recorded with a "cut-off" of 120s, every hour for 5 h.

Comparisons between groups were made using a one-way ANOVA procedure followed by a post hoc least square means multiple comparison test using SAS Proc GLM. A probability of $P < 0.05$ was considered significant. ED$_{50}$ values and 95% confidence limits were calculated using a logistic log dose linear regression analysis using SAS Proc REG.

Electrophysiological Recordings

Male Sprague-Dawley rats (280–330 g) were anesthetized with chloral hydrate (400 mg/kg i.p.); supplemental doses of anesthetic were administered through the lateral tail vein as needed. Body temperature was maintained at 37°C by a heating pad. The anesthetized rats were mounted in a stereotaxic apparatus, the skull was exposed, and a cisternal drain was performed to prevent tissue swelling. A burr hole was made in the skull over the A9 and A10 areas. Electrodes were fabricated from 2.0-mm capillary tubing (Radnoti, Monrovia, CA) on a Narishige (Tokyo, Japan) electrode puller (model PE-2). Electrodes were filled with a 2 M NaCl solution and broken back to impedances of 2.0 to 3.0 MΩ.

The tip of the recording electrode was lowered to the dorsal border of either A9 or A10 and then advanced using a micropositioning device (Inchworm Motor Controller; Burleigh, Victor, NY) in 5-μm increments through the nucleus. The electrode was passed through nine tracks (each track was separated by 0.2 mm) in a stereotaxically defined block of tissue (5.0–5.4 mm posterior, 2.0–2.4 mm lateral to bregma, 6.0–8.5 mm ventral to the cortical surface for A9; and 5.0–5.4 mm posterior, 0.5–0.9 mm lateral to bregma, and 6.0–8.5 mm ventral to the cortical surface for A10), and the number of spontaneously active dopamine cells was counted. The electrode tracks were made in a preset sequence that was kept constant from animal to animal. For acutely treated animals, three control tracks were scored before drug treatment. Six additional tracks were then recorded 1 h after s.c. injection of FMPD or vehicle. For chronically treated animals, osmotic minipumps (Alzet model 2ML1; Durect Corp., Cupertino, CA) delivering vehicle or 10 mg/kg/day FMPD were implanted under isoflurane (5%) anesthesia. Only one area, either A9 or A10, was recorded in each animal. Spontaneously active dopamine cells were recorded as reported previously (Chiodo and Bunney 1983; Stockton and Rasmussen, 1996). Briefly, cells were considered dopaminergic if they possessed the following characteristics: 1) action potential duration of 2.5 to 4.5 ms; 2) triphasic wave form containing a notch in the initial rising phase of the first positive peak; and 3) slow slightly irregular firing with a rate of 2 to 10 Hz. Previously, these characteristics have been demonstrated to be shown only by dopaminergic neurons (Bunney et al. 1973). Activity from the electrode was bandpass-filtered (300–3000 Hz; Dagan model 2400, Minneapolis, MN), and passed through a Micro1401 data acquisition unit connected to a PC running Spike2 software (Cambridge Electronic Design, Cambridge, UK). The firing rate of each cell was monitored for 1 to 2 min to insure that the cells had not been mechanically excited.

Fos Immunohistochemistry

Tissue Preparation. Male Sprague-Dawley rats weighing 155 to 175 g were housed in groups of four in a 22°C room with lights on from 7:00 AM to 7:00 PM for 1 week before experimentation. Food and water were freely available. Olanzapine (Eli Lilly & Co.) and FMPD were prepared to the required concentration in vehicle solution (0.4% lactic acid) and injected s.c. Control rats received vehicle injections. Animals ($n = 4–6/group) were sacrificed by decapitation 2 h after vehicle or drug administration. The brains were rapidly removed, frozen in isopentane (2-methyl butane), cooled on dry ice, and stored at −80°C until sectioned. Coronal sections through the nucleus accumbens (+1.0 mm relative to bregma), and lateral hypothalamus (−2.80 mm relative to bregma) were cut at 12 μm in a cryostat and thaw-mounted onto Superfrost Plus slides. Sections were allowed to dry completely and were stored at −20°C until processed. Immediately before staining, slides were fixed in a 4% paraformaldehyde solution containing phosphate-buffered saline (pH 7.4) for 10 min.

Fos Profile Immunohistochemistry. After fixation, slides were stained for Fos-like immunohistochemistry as described previously (Sundquist and Nisenbaum, 2005). In brief, slides were washed in wash buffer (0.05 M Tris-HCl, 0.15 M NaCl, and 0.05% Tween 20) and placed in 0.3% hydrogen peroxide in methanol for 15 min to quench endogenous peroxidase. The slides were washed in buffer and incubated in Powerblock (Biogenex, San Ramon, CA) for 5 min. Sections were incubated in goat anti-Fos antibody (1:2000; Santa Cruz SC52G; Santa Cruz Biotechnology, Santa Cruz, CA) for 90 min in antibody diluent (1% fatty acid-free bovine serum albumin in wash buffer) followed by several washes in buffer. Sections were then incubated in a biotinylated horse anti-goat secondary antibody (1:200; Vector Laboratories, Burlingame, CA) in antibody diluent followed by several washes in buffer. Sections were then reacted with avidin-biotin complex (Vectastain Elite; Vector Laboratories) for 30 min followed by several washes in buffer. Slides were then incubated in a biotinylated horse anti-goat secondary antibody (1:200; Vector Laboratories, Burlingame, CA) in antibody diluent followed by several washes in buffer. Slides were then reacted with avidin-biotin complex (Vectastain Elite; Vector Laboratories) for 30 min followed by several washes in buffer. The slides were then dehydrated through graded alcohols, cleared in Citrasolve (Fisher Scientific Co., Pittsburgh, PA) and coverslipped with Vectamount (Vector Laboratories). Sections were identified by a numbered key, and counts were carried out blinded to treatment. Fos-positive cells were counted using the image analysis software MCID Elite 6.0 (Imaging Research, St. Catharines, ON, Canada). The number of Fos-positive cells was counted within a 300 × 320-μm box in the dorsolateral striatum and the shell of the nucleus accumbens. Counts were taken on the left and right sides of two sections per animal.

The atypical index, based on the methods of Robertson et al. (1994), was calculated for each group using the following formula: Atypical Index = (NAcD − NAcV) − (DLStD − DLStV) where NAcD =...
number of Fos-positive cells in the nucleus accumbens shell produced by antipsychotic drug, NAcF = number of Fos-positive cells in the nucleus accumbens shell produced by vehicle, DLSTp = number of Fos-positive cells in the dorsolateral striatum produced by antipsychotic drug, and DLSTv = number of Fos-positive cells in the dorsolateral striatum produced by vehicle.

**Fos/Orexin Double-Label Immunohistochemistry.** After fixation, slides were washed in wash buffer (0.05 M Tris-HCl, 0.15 M NaCl, and 0.05% Tween 20) and incubated in Powerblock for 5 min. Sections were then incubated for 90 min in a cocktail of rabbit anti-Fos antibody (1:1000; Santa Cruz SC52) and goat anti-orexin A antibody (1:750; Santa Cruz SC8070; Santa Cruz Biotechnology) in antibody diluent (1% fatty acid-free bovine serum albumin in wash buffer) followed by several washes in buffer. Sections were then incubated for 30 min in a cocktail of biotinylated donkey anti-rabbit secondary antibody (1:300; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and fluorescein isothiocyanate donkey anti-gold secondary antibody (1:150; Jackson ImmunoResearch) in antibody diluent followed by several washes in buffer. Sections were then incubated for 30 min in a cocktail of streptavidin conjugated to Alexa 594 (1:1600; Invitrogen, Carlsbad, CA) and a goat anti-fluorescein isothiocyanate antibody (1:300; Invitrogen) in antibody diluent followed by several washes in buffer. Sections were then incubated for 30 min in a cocktail of streptavidin conjugated to Alexa 594 (1:1600) and a donkey anti-gold antibody conjugated to Alexa 488 (1:200; Invitrogen) in antibody diluent followed by several washes in buffer. The slides were then overlaid with fluorescent mounting medium (DakoCytomation California Inc., Carpinteria, CA) and stored in the dark at 4°C. The slides were identified by a numbered key, and counts were carried out blinded to treatment. The slides were photographed using a Spot RT camera (Diagnostic Products, Los Angeles, CA) mounted on a Leica DMR fluorescence microscope. Cells in the lateral hypothalamus (lateral to the fornix) exhibiting both Fos and orexin immunoreactivity were counted, along with the total number of orexin-immunoreactive cells on the left and right sides of four sections per animal. These numbers were used to calculate the percentage of orexin cells exhibiting Fos expression.

**Statistical Evaluations.** The data were analyzed using one-way ANOVA followed by Tukey’s (JMP 4.0.4 software; SAS Institute, Cary, NC). The level of significance was set at *, p < 0.05.

**Prolactin Levels**

Male Fisher 344 (Harlan) weighing between 180 and 240 g were maintained on a 12-h light/12-h dark schedule (lights on at 6:00 AM) and fed Rodent Diet 5002 (PMI Nutrition International, Inc, Richmond, IN) and water ad libitum. An indwelling atrial cannula was placed in each rat via the external jugular vein under isoflurane anesthesia. The incision was closed using three surgical wound clips. Antibiotic ointment was applied, and the animal was observed until full ambulation was apparent. After recovery, animals were weighed and then returned to their home cage.

After surgery, the animals received standard laboratory chow ad libitum, and the weight of the animals was measured on days 3, 7, 10, and 14 after pump insertion. Weight gain of the drug- and vehicle-treated groups of FMPD was compared using a two-way ANOVA with repeated measures analysis, and individual differences were measured using a post hoc Tukey’s analysis. Weight gain of the drug- and vehicle-treated groups for olanzapine, risperidone, and haloperidol were compared using a one-tailed Student’s t test for significance.

FMPD and olanzapine HCl were dissolved in dH₂O with 1 μl of 10% dilute lactic acid for every 10 mg of drug. Once dissolved, the pH was adjusted to 4 before loading into minipumps. Risperidone and haloperidol were dissolved in dH₂O.

**Results**

**Radioligand Binding.** The affinity of FMPD and olanzapine for 34 different monoaminergic and muscarinic neurotransmitter receptors is shown in Table 2.

FMPD and olanzapine showed similar affinities for most of the receptors examined. This is illustrated in Fig. 2, where it can be seen that most of the receptor affinities for the two compounds lie close to the line of identity for the comparison (i.e., the absolute affinities are quite close). Only a few receptors showed an affinity difference of greater than or equal to 5-fold, and these are highlighted in Fig. 2. These included the human recombinant forms of the dopamine D₁, histamine H₁, α₁-adrenergic, and muscarinic M₁ receptors as well as the rat histamine H₁ and muscarinic (subtype unspecified) receptors.

**Inhibition of Ex Vivo Binding to 5-HT₆ and Histamine H₁ Receptors.** Both FMPD (ED₅₀ = 10 mg/kg) and olanzapine (ED₅₀ = 4 mg/kg) inhibited ex vivo binding to 5-HT₆ receptors. Olanzapine inhibited histamine H₁ receptor binding (ED₅₀ = 0.7 mg/kg), but FMPD did not inhibit histamine H₁ receptor binding up to 30 mg/kg (Fig. 3).

**Nucleus Accumbens DOPAC Concentrations.** Administration of FMPD caused a significant elevation of nucleus accumbens DOPAC levels (ED₂₀₀ = 6 mg/kg), as did olanzapine (ED₂₀₀ = 1.3 mg/kg; Fig. 4).

**Serum Corticosterone Concentrations.** Administration of FMPD blocked quipazine-induced increases in rat serum corticosterone levels (ED₁₈₀ = 1.8 mg/kg), as did olanzapine (ED₁₈₀ = 2 mg/kg; Fig. 5).

**Cocaine-Induced Locomotor Activity.** Cocaine (40 mg/kg i.p.) produced a marked increase in activity, the peak effect occurring after 40 to 60 min, and the activity then declined over the 90-min test period (Fig. 6). FMPD (1.25–10 mg/kg p.o.) and olanzapine (1.25–10 mg/kg p.o.) (Fig. 6) sig-
significantly reduced the peak hyperactivity with ED$_{\text{min}}$ of 2.5 (FMPD) and 1.25 mg/kg p.o. (olanzapine).

**Catalepsy.** FMPD produced a dose-related increase in catalepsy (ED$_{\text{min}}$ 2.5 mg/kg p.o.; Fig. 7). Olanzapine (6.25–50 mg/kg p.o.) produced catalepsy at the highest dose tested (50 mg/kg p.o.). Olanzapine (6.25–50 mg/kg p.o.) significantly reduced the peak hyperactivity with ED$_{\text{min}}$ of 2.5 mg/kg p.o. (Fig. 7).

Table 3 compares the catalepsy ED$_{\text{min}}$/cocaine hyperactivity ED$_{\text{min}}$ ratio for FMPD, olanzapine, haloperidol, and chlorpromazine. The ratio for FMPD is 5- to 10-fold greater than that observed for the older typical antipsychotics, haloperidol, and chlorpromazine, but it is 4-fold less than olanzapine.

**Electrophysiology.** Acute administration of FMPD (10 mg/kg s.c.) did not change the number of spontaneously active A9 dopamine neurons but did cause a significant elevation of the number of spontaneously active A10 dopamine neurons (Fig. 8). Chronic administration of FMPD (10 mg/kg/day s.c. for 21 days) did not change the number of spontaneously active A9 dopamine neurons but did cause a significant decrease in the number of spontaneously active A10 dopamine neurons (Fig. 8).

**Fos Induction.** Fig. 9 shows the effects of olanzapine (A) and FMPD (B) on regional Fos expression. The atypical index for olanzapine was positive for both the 1 and 5 mg/kg doses (9.9 and 17.85, respectively). Likewise, the atypical index for FMPD was positive for both the 10 and 20 mg/kg doses (15.47 and 26.35, respectively).

As shown in Table 4, the percentage of orexin-containing cells of the lateral hypothalamus that exhibit c-Fos induction in response to olanzapine is significantly higher than that seen in vehicle animals. Similar results were seen for FMPD, suggesting that it may show similar weight gain propensity to olanzapine.

**Prolactin Elevation.** Treatment with FMPD at doses of 1, 5, and 10 mg/kg produced a rapid but transient elevation in prolactin secretion (Fig. 10). Peak mean circulating levels were reached at the first sample time (20 min). By 60 min, prolactin levels had returned to baseline in the 1 and 5 mg/kg group. At a dose of 10 mg/kg, plasma prolactin levels were slightly but significantly elevated at 3 h.

**Weight Gain.** Significant weight gain was observed over time with the continuous administration of FMPD (Fig. 11A). Although no significance was reached on day 3 ($F = 2.65; p = 0.06$), more weight gain was observed in the animals treated with FMPD (11–15 g) than those treated with vehicle (7 g). On day 7, there was a significant treatment effect ($F = 5.6, p < 0.003$) and 5 mg/day was significantly different from vehicle. In fact, more weight gain was observed with the 5 mg/day dose compared with vehicle across the entire experiment after day 3. Peak weight gain with FMPD was approximately 25 g over the 14 days. Greater weight gain than vehicle was also observed with the dose of 3 mg/day, but significance was not reached because of group variability around the mean. The treatment means at days 10 and 14 ($F = 9.66, p < 0.0002; F = 3.81, p < 0.02$) also revealed a treatment effect of FMPD.
Likewise, weight gain was also observed in female rats with olanzapine (Fig. 11B), risperidone (Fig. 11C), and haloperidol (Fig. 11D). Significant weight gain was observed with olanzapine (1.75 mg/day; up to 25 g over 14 days), with separation from vehicle beginning on day 7. Significant weight gain was also observed with risperidone (0.5 mg/day; peak effect of approximately 35 g) beginning at day 7. Significant weight gain was also observed with haloperidol (0.05 mg/day; up to 35 g over 14 days), with a significant effect beginning at day 7.

**Discussion**

FMPD has high affinity for $D_2$ receptors and is a potent $D_2$ antagonist in vivo. $D_2$ receptor antagonists increase the release of dopamine into the synapse due to blockade of the $D_2$ autoreceptor (Karoum et al., 1994). This increased release of dopamine cannot be directly measured, because the efficiency of the dopamine reuptake system prevents increases in synaptic dopamine concentrations. Instead, increases in the levels of the dopamine metabolites DOPAC and homovanillic acid reflect increased neuronal dopaminergic activity in vivo. For example, olanzapine and other dopamine $D_2$ receptor antagonists increase concentrations of DOPAC and homovanillic acid in striatum and nucleus accumbens without appreciable alteration of dopamine concentrations (Karoum et al., 1994; Bymaster et al., 1996). Oral administration of FMPD increased rat nucleus accumbens DOPAC concentrations ($ED_{200} = 6$ mg/kg). Under similar conditions,
olanzapine increased rat nucleus accumbens DOPAC concentrations (ED\textsubscript{200} = 1.3 mg/kg). Because all currently used antipsychotics are antagonists (aripiprazole seems to be a weak partial agonist under some conditions; Burris et al., 2002) at the D\textsubscript{2} receptor and the clinical potencies of many of these antipsychotics are directly related to their D\textsubscript{2} affinity (Seeman et al., 1976), it is likely that FMPD has the potential for antipsychotic properties in humans.

FMPD also has high affinity for 5-HT\textsubscript{2A} receptors and is a potent 5-HT\textsubscript{2A} antagonist in vivo. 5-HT is one of many neurotransmitters that influence hypothalamic control of pituitary function. Direct-acting 5-HT\textsubscript{1A} or 5-HT\textsubscript{2A} receptor agonists increase serum corticosterone concentrations in rats by activation of corticotropin-releasing factor-containing neurons in the paraventricular nucleus of the rat hypothalamus that stimulate release of adrenocorticotropic hormone from the anterior pituitary, which releases corticosterone from the adrenal cortex (Tuomisto and Mannisto, 1985). Increases in corticoid levels produced by 5-HT\textsubscript{2A} receptor agonists like quipazine are dose dependently blocked by selective 5-HT\textsubscript{2A} receptor antagonists (Hemrick-Luecke and Evans, 2002). Oral administration of FMPD blocked quipazine-induced increases in rat serum corticosterone levels (ED\textsubscript{50} = 2 mg/kg). Under similar conditions, olanzapine also blocked quipazine-induced increases in rat serum corticosterone levels (ED\textsubscript{50} = 2 mg/kg). Most atypical antipsychotics have a high affinity for the 5-HT\textsubscript{2A} receptor, and many have been shown to occupy 5-HT\textsubscript{2A} receptors at clinically relevant doses (Kapur et al., 1999). It has been hypothesized that a high affinity for the 5-HT\textsubscript{2A} receptor helps treat the negative symptoms of schizophrenia and prevent some of the motor side effects (Meltzer et al., 1989). However, selective 5-HT\textsubscript{2A} antagonists are not effective antipsychotics as monotherapy (Marder, 1999) and 5-HT\textsubscript{2A} antagonists do not modify the catoleptogenic effects of D\textsubscript{2} antagonists (Kalkman et al., 1998). Thus, the exact role of antagonism of the 5-HT\textsubscript{2A} receptor in the clinical effects of antipsychotics remains to be further clarified.

Unlike most other antipsychotics, olanzapine and clozapine have high affinity for the 5-HT\textsubscript{6} receptor (Bymaster et al., 1999). FMPD also has high affinity for the 5-HT\textsubscript{6} receptor and inhibited the ex vivo binding of the 5-HT\textsubscript{6} ligand \([\text{\textsuperscript{125}I}]\text{SB-258585}\) to striatal 5-HT\textsubscript{6} receptors (ED\textsubscript{50} = 10 mg/kg). Because olanzapine and clozapine display good efficacy in treating the cognitive disturbances of schizophrenia (Purdon et al., 2000; Bilder et al., 2002) and selective 5-HT\textsubscript{6} antagonists are active in models of cognitive enhancement...
(King et al., 2004), FMPD may also show efficacy in treating the cognitive disturbances of schizophrenia. However, risperidone also has some efficacy in the cognitive disturbances of schizophrenia but has low affinity for the 5-HT6 receptor (Bilder et al., 2002). Thus, although 5-HT6 antagonist activity may contribute to treating cognitive disturbances in schizophrenia, it may not be necessary.

One problematic adverse event observed with many antipsychotics is weight gain (Allison et al., 1999). Affinity for the H1 receptor has been proposed as a hypothesis for the mechanism of the treatment-emergent weight gain. Histamine activates postsynaptic H1 receptors in the ventromedial and para-ventricular nucleus of the hypothalamus to suppress feeding (Sakata et al., 1988; Ookuma et al., 1989), and for antipsychotics, there is a significant correlation between affinity for the human H1 receptor and weight gain (Kroeze et al., 2003). FMPD has 8-fold lower affinity for the human H1 receptor compared with olanzapine. Ex vivo binding studies in rats show that FMPD had no measurable activity at the H1 receptor up to 30 mg/kg p.o., whereas olanzapine had significant activity at H1 receptors at relatively low doses (ED50 = 0.8 mg/kg p.o.). Because FMPD has 2-fold higher affinity for the human H1 receptor compared with the rat H1 receptor, it may have slightly more activity at human H1 receptors than is indicated by the rat ex vivo data. However, FMPD has 5-fold lower affinity for H1 receptors compared with D2 receptors, whereas olanzapine has 3-fold higher affinity for the H1 receptor compared with the D2 receptor. Thus, FMPD is likely to have significantly less activity at H1 receptors in

### Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg s.c.)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (for olanzapine)</td>
<td>11.64 ± 0.56</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>1</td>
<td>43.77 ± 1.19*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>48.02 ± 0.82*</td>
</tr>
<tr>
<td>Vehicle (for FMPD)</td>
<td>13.48 ± 0.84</td>
<td></td>
</tr>
<tr>
<td>FMPD</td>
<td>10</td>
<td>47.97 ± 0.96*</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>51.53 ± 0.62*</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. vehicle.
humans compared with olanzapine. This decreased activity at H₁ receptors compared with olanzapine may also improve the effects of FMPD on the cognitive symptoms of schizophrenia, because H₁ antagonists have been shown to decrease cognitive performance (Okamura et al., 2000). In female rats, the weight gain observed with FMPD treatment was equivalent to the weight gain observed with olanzapine treatment (Fig. 11); however, the clinical relevance of this rodent weight gain is unclear, as significant weight gain was also observed with both haloperidol and risperidone (Fig. 11; Fell et al., 2004), two compounds that have been reported to have less treatment-emergent weight gain than olanzapine (Kroeze et al., 2003).

Although H₁ activity may explain some of the weight gain observed with antipsychotic treatment, other receptors are likely to play a role. For example, quetiapine has greater than 20-fold higher affinity for H₁ receptors compared with D₂ receptors (Kroeze et al., 2003). Whereas weight gain is observed with quetiapine treatment, it has been reported to have less treatment-emergent weight gain than olanzapine (Kroeze et al., 2003). Thus, antagonism of H₁ receptors may not fully account for all of the treatment-emergent weight gain observed with antipsychotic use. Another receptor that may contribute to treatment-emergent weight gain is the 5-HT₂C receptor. 5-HT₂C receptor knock-out mice gain more weight than control mice (Tecott et al., 1995). This phenomenon is age-dependent, beginning in “middle aged” mice. In addition, 5-HT₂C antagonists cause increased eating and weight gain (see Dourish, 1995). In this regard, low affinity of quetiapine for 5-HT₂C receptors may decrease its propensity for treatment-emergent weight gain. FMPD has somewhat lower affinity for 5-HT₂C receptors than olanzapine. Thus, because of its lower affinity for both H₁ and 5-HT₂C receptors compared with olanzapine, FMPD may have less treatment-emergent weight gain. Only clinical trials can test this hypothesis.

Whereas the binding affinity of FMPD indicates that it has the potential for less treatment-emergent weight gain than olanzapine, one preclinical assay predicts that it may have similar weight gain observed with its treatment compared with olanzapine. Antipsychotic drugs that have treatment-emergent weight gain have been shown to increase Fos expression in a high percentage of orexin-containing neurons of the lateral hypothalamus (Fadel et al., 2002). To help examine the potential for treatment-emergent weight gain, we compared the effects of olanzapine and FMPD on Fos expression in orexin neurons. Similar to a previous study (Fadel et al., 2002), olanzapine produced a significant increase in the percentage of orexin cells that also exhibited Fos activity. In addition, the percentage of double-labeled orexin neurons was similar between treatments with FMPD and olanzapine. These data suggest that FMPD and olanzapine may have a similar degree of weight gain observed with their treatment. However, only a limited number of compounds have been examined in this Fos assay, thus its predictive validity remains unclear.

Another important property of atypical antipsychotics is their more favorable EPS profile. Both FMPD and olanzapine blocked cocaine-induced locomotor activity (confirming their activity as a dopamine antagonist in vivo), and both FMPD and olanzapine induce catalepsy; however, olanzapine was less active than FMPD. The ratio of the effective dose in catalepsy over an effective dose as a dopamine D₂ antagonist (in this case catalepsy ED₉₀ and the dopamine hyperactivity ED₉₀), is one measure of the margin of safety between the efficacious dose and the dose likely to induce EPS of a compound (Moore, 1999). This ratio for FMPD is greater than that observed for the older atypical antipsychotics haloperidol and chlorpromazine but somewhat smaller than olanzapine. These data indicate that FMPD is likely to have a lower potential for EPS than typical antipsychotics.

Other preclinical predictors of treatment-emergent EPS include the effects of compounds on midbrain dopamine unit activity. Acute administration of typical antipsychotic drugs (e.g., haloperidol) increase and chronic administration decreases the number of spontaneously active dopamine cells in both the ventral segmental area (A10; which projects primarily to the limbic and cortical regions) and the substantia nigra pars compacta (A9; which projects primarily to the striatum) (Chiodo and Bunney, 1983). However, acute administration of atypical antipsychotics (e.g., clozapine) selectively increases and chronic administration selectively decreases the number of spontaneously active A10 but not A9 dopamine cells (Chiodo and Bunney, 1983). Thus, atypical antipsychotics have selective effects on A10 versus A9 dopamine neurons. Therefore, since atypical antipsychotics have a greatly reduced propensity for producing treatment-emergent EPS, it has been hypothesized that the effects on A10 activity may underlie the therapeutic effects of antipsychotics, whereas the effects on A9 activity may underlie the EPS associated with typical antipsychotic treatment. We have shown previously that olanzapine produces a selective effect on A10 dopamine neurons after both acute and chronic administration (Stockton and Rasmussen, 1996). Similar to olanzapine and other atypical antipsychotics, acute administration of 10 or 20 mg/kg s.c., FMPD produced a significant elevation in the number of spontaneously active A10 neurons but did not change the number of spontaneously active A9 neurons (Fig. 8). Also characteristic of atypical antipsychotics, chronic administration of 10 mg/kg s.c. FMPD produced a significant decrease in the number of spontaneously active A10 neurons but did not change the number of spontaneously active A9 neurons (Fig. 8). The selective effects of FMPD on A10 dopamine neurons predict that it will be an atypical
antipsychotic, similar to olanzapine, and have a low propensity for treatment-emergent EPS.

Another preclinical model predicting the potential for treatment-emergent EPS is the Fos-like immunoreactivity (Robertson et al., 1994). Administration of antipsychotic drugs in rats leads to an induction of Fos-like immunoreactivity in the brain. Typical antipsychotics, such as haloperidol, greatly increase Fos expression in several regions of the brain including the dorsolateral striatum and nucleus accumbens, whereas atypical antipsychotics produce an increase in the nucleus accumbens but very little if any change in the dorsolateral striatum. Any drug that produces a positive value for the Atypical Index is predicted to act as an atypical antipsychotic, whereas a negative number is indicative of a typical antipsychotic. Consistent with previous studies (Robertson and Fibiger, 1996), the atypical index for olanzapine was positive for both the 1 and 5 mg/kg doses (9.9 and 17.85, respectively), indicating that olanzapine is an atypical antipsychotic as defined by this criterion. Likewise, the atypical index for FMPD was positive for both the 10 and 20 mg/kg doses (15.47 and 26.35, respectively), indicating that FMPD is predicted to be an atypical antipsychotic as defined by this criterion and that minimal treatment-emergent EPS will be observed in humans.

Antipsychotics produce an elevation in circulating prolactin levels in rats and humans (Kapur et al., 2002; Kinon et al., 2003). The prolactin elevation in patients for olanzapine is lower in magnitude and more transient compared with

---

**Fig. 11.** A, effect of FMPD on weight gain over 14 days in female rats. Each point represents the mean (± S.E.M.) change from day 0 for five rats (○, vehicle; ▲, 1 mg/day; ▼, 3 mg/day; ▣, 5 mg/day). *, significant from vehicle-treated animals on any specific treatment day, *p* < 0.05. B, effect of olanzapine on weight gain over 14 days in female rats. Each point represents the mean (± S.E.M.) change from day 0 for six rats (○, vehicle; △, 1.75 mg/day). *, significant from vehicle-treated animals on any specific treatment day, *p* < 0.05. C, effect of risperidone on weight gain over 14 days in female rats. Each point represents the mean (± S.E.M.) change from day 0 for five rats (○, vehicle; □, 0.5 mg/day). *, significant from vehicle-treated animals on any specific treatment day, *p* < 0.05. D, effect of haloperidol on weight gain over 14 days in female rats. Each point represents the mean (± S.E.M.) change from day 0 for five rats (○, vehicle; ▼, 0.05 mg/day). *, significant from vehicle-treated animals on any specific treatment day, *p* < 0.05.
sustained elevation of haloperidol and risperidone (Crawford et al., 1997; Turrone et al., 2002). After acute administration, FMPD produced a transient elevation in rat prolactin levels (Fig. 10) despite maintaining high plasma levels (J. T. Catlow and S. Swanson, unpublished observations) over this time period. The transient prolactin elevation produced by FMPD is similar to the transient prolactin elevation produced by olanzapine and different from the sustained prolactin elevation produced by risperidone in rats (Rourke et al., 2004). The plasma prolactin data from the acute rat study indicates that FMPD has the potential to have a modest effect on prolactin secretion in patients.

During treatment with some atypical antipsychotics, new-onset diabetes mellitus has been observed (Henderson, 2002). This is a controversial issue as studies have shown that first-episode drug-naive schizophrenic patients have impaired fasting glucose tolerance, are more insulin-resistant, and have higher levels of plasma glucose, insulin, and cortisol than healthy controls (Ryan et al., 2003). In addition, three weeks of treatment with olanzapine or risperidone did not result in significant changes to insulin sensitivity in healthy subjects (Sowell et al., 2003). Two recent studies have implicated antagonism at the cholinergic muscarinic M3 receptor as a possible contributing factor to new-onset diabetes in schizophrenic patients treated with antipsychotics (Johnson et al., 2005; Silvestre and Prous, 2005). FMPD has very low affinity for human cholinergic muscarinic M3 receptors (Kᵦ = 1935 nM; Table 2). Thus, clinical trials with FMPD could help shed light on the cholinergic muscarinic M3 hypothesis of new-onset diabetes in schizophrenia patients treated with antipsychotics.

In conclusion, FMPD is a potential novel atypical antipsychotic with in vivo antagonist activity at dopamine D2, 5-HT₂A, and 5-HT₆ receptors but with lower affinity for histamine H₁ and 5-HT₃ receptors than olanzapine. One behavioral measure of its activity predicts that FMPD will have more treatment-emergent EPS than olanzapine, whereas electrophysiology and Fos-induction assays predict that treatment-emergent EPS, similar to olanzapine, will be observed. The lower affinity of FMPD for H₁ and 5-HT₃ receptors predicts that it will have less weight gain observed with its treatment than olanzapine; however, a Fos-induction assay predicts that it will have weight gain observed with its treatment similar to olanzapine. Only clinical trials with FMPD will be able to address these discrepant predictions and determine its clinical utility. The results of these clinical trials will, in turn, tell us more about the predictive validity of current preclinical models for antipsychotic efficacy and adverse events.

Acknowledgments

We acknowledge the contributions of Dr. Neil Delapp, David C. Evans, Brian Getman, Judi M. Graham, Virginia L. Luicates, Dr. Kevin L. McKnight, Dr. W. Martin Owton, Dr. Lee Phebus, Dr. Marta M. Pineiro-Nunez, Dr. Vincent P. Rocco, Penny Thralkeld, Bradley Wainscott, Yili Yang, and Ting-gui Yin to the completion of this manuscript.

References

Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (Kᵦ) and the concentration of inhibitor which causes 50 per cent inhibition (I₅₀) of an enzyme reaction. Biochem Pharmacol 22:3099–3108.
5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation—an effect sensitive to NMDA receptor antagonism. *Neuropharmacology* **47**:185–204.


**Address correspondence to:** Dr. Kurt Rasmussen, Lilly Research Laboratories, Eli Lilly and Co., Lilly Corporate Center, Indianapolis, IN 46285.

E-mail: rasmussen_kurt@lilly.com